Iranian Journal of Veterinary Science and Technology
Vol. 2, No. 1, 2010, 17-24

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Peritoneal Fluid Cytology in Clinical Cases of Bovine Obstructive Urolithiasis

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Received: December 1, 2009 Accepted: May 5, 2010

Abstract

Thirty clinical cases of obstructive urolithiasis, 15 with intact urinary bladder and 15 with ruptured urinary bladder, were used to study the changes in peritoneal fluid cytology. The percentage of neutrophils was highly increased than the normal reference range reported for cattle with slight decrease in lymphocyte percentage. The value for neutrophil percent was almost similar in both intact and ruptured urinary bladder cases. The monocyte/mesothelial cells/macrophage percent, though increased than the normal reference range were identical in both the groups. There was a predominant decrease in eosinophil percentage with more decrease in intact urinary bladder cases. Polymorphonuclear-to-mononuclear cell ratio was same in both the groups, but higher than the normal reference value (1:1). Eosinophils ≤ 8% and neutrophil ≥ 30% could be established as a reference range of peritonitis in calves. Red blood corpuscles (RBCs) were totally absent in the peritoneal fluid samples obtained from intact urinary bladder cases except in one case. However, in the peritoneal fluid samples obtained from ruptured urinary bladder cases, RBCs were found in abundance in 3 cases and very few in 2 cases. The morphology and the different cell type present in the peritoneal fluid samples also varied according to the status of urinary bladder. In intact urinary bladder cases, neutrophils were mature and nondegenerate, while in ruptured urinary bladder cases degenerate and hypersegmented neutrophils were more. Mesothelial cells were equally distributed in both the groups. Bacteria were found extracellularly as well as intracellularly in degenerate neutrophils in 4 cases with ruptured urinary bladder and only extracellularly in 2 cases with intact urinary bladder. Peritoneal fluid cytology can be used for differentiation of peritonitis from normal cases and non septic peritonitis from septic peritonitis besides diagnosing the uroperitoneum in calves.

Keywords: calves, cytology, peritoneal fluid analysis, uroperitoneum, urolithiasis

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Introduction

Peritoneal fluid analysis has considerable potential as an aid to the haematological and clinical examination in the diagnosis of bovine abdominal disorders, since it can indirectly assess the volume of peritoneal fluid, cellularity and protein characteristics thereby giving an indication of inflammatory changes in the peritoneal cavity (Oehme, 1969; Oehme and Noordsy, 1970). Cytological examination of peritoneal fluid can help distinguish among fungal, bacterial, sterile inflammatory or neoplastic causes of effusion, and often assists the clinician in initiating appropriate therapy while waiting for the results of culture, antimicrobial susceptibility testing, and histological examination (Wilson *et al*., 1985; Roussel and Ward, 1985; Hirsch and Townsend, 1982; Oehme, 1969; Bohn and Callan, 2007). Peritoneal fluid in adult cattle has great variability. Peritoneal fluid constituents can be affected by stage of pregnancy, disease, and location of the lesion with respect to the site of peritoneal fluid collection (Anderson *et al*., 1995; Radostits *et al*., 2000). Peritoneal fluid analysis has been reported for cattle with displacement of the abomasum, metritis, omental bursitis, peritonitis, and ascites (Oehme 1969; Wilson *et al*., 1985; Kopcha and Schultzc, 1991; Hirsch and Townsend, 1982). The peritoneal fluid constituents of clinically normal young calves have been described (Anderson *et al*., 1995); however, no detailed information regarding the change in peritoneal fluid constituents in calves suffering from complete obstructive urolithiasis is available. Urolithiasis may affect any species of the animals but is considered of great economic importance in fattening steers being fed heavy concentrate ration and in castrated lambs (Bhatt *et al*., 1973; Radostits *et al.,* 2000). Urolithiasis has been reported more frequently in feed lot and grazing cattle in Western America (Monoghan and Boy, 1990). In India, urolithiasis has been commonly reported in bullocks (Gera *et al*. 1973; Ashturkar, 1994), while in Kashmir valley urolithiasis is most prevalent among cow calves below 1 year of age (Muhee, 2006).This study was thus undertaken to record the changes in peritoneal fluid cellularity of calves suffering from obstructive urolithiasis.

Materials and Methods

Thirty male cattle calves suffering from complete obstructive urolithiasis, fifteen with intact urinary bladder and 15 were with ruptured urinary bladder presented for treatment at Teaching Veterinary Clinical Services Complex, Faculty of Veterinary Sciences and Animal Husbandry (F. V. Sc & A. H.), Sher - e - Kashmir University of Agricultural Sciences and Technology of Kashmir (SKUAST-K), Srinagar, formed the material of the study. At the time of admission, all the animals were subjected to complete pre-operative clinical, radiographic, sonographic and haematobiochemical examination. Physical and cytological examination of peritoneal fluid and cytological examination of blood was carried out.

Abdominocentesis was performed slightly lateral and caudal to the umbilicus to obtain peritoneal fluid. The animals were restrained in left lateral recumbent position with right hind limb pulled dorsally and caudally. The sites were prepared aseptically and not infiltrated with any local anesthetic. A 20 gauge 5cm hypodermic needle mounted over a 5ml disposable sterile syringe was introduced and directed slightly caudally and toward the midline while maintaining it parallel to the internal abdominal wall once the peritoneal cavity was entered. Gentle suction was applied and the peritoneal fluid obtained was placed in a 2ml tube containing tri-potassium EDTA. After completing the physical examination, the peritoneal fluid was centrifuged at 1500 g for 10 minutes. The supernatant was preserved at -20^0c until further analysis. The sediment was used for preparing a smear by routine procedure. The peritoneal fluid smears were air dried, stained with Wrights stain and examined for cell differential count and cellular morphology. RBCs count was

performed per high power field. Oil immersion was used for recording the presence of bacteria, cell morphology and other substances, if present. Differential cell counts and cytological findings were made especially at the end of the slide and at the edges of the slide, where abundance of the cells was found. Four categories of nucleated cells were reported, neutrophils, lymphocytes, monocytes and other similar mononuclear cells, and eosinophils. Large mononuclear cells, including macrophages, activated macrophages and mesothelial cells were all grouped together as monocytes.

For haematological studies, 2ml of blood was collected from clinical cases of obstructive urolithiasis from jugular vein using aseptic syringes and collected in sterile heparinised vials.

The data thus obtained was classified and subjected to t test using SPSS 12.0 software and inferences drawn.

Results

The peritoneal fluid differential cell count is represented in the Table.

The overall neutrophil percentage was highly increased and lymphocyte percentage slightly decreased in PF of clinical cases of obstructive urolithiasis than the normal reference range reported for cattle. The mean ± SE neutophil percent was 30.48 ± 1.44% (27 - 46%), 33.08 ± 1.39% (27 - 40%), and 33.50 ± 1.48% (27 - 46%) in whole group, intact urinary bladder and ruptured urinary bladder cases respectively. The neutrophil percent was almost similar in both the intact and ruptured urinary bladder cases. The value for lymphocyte percent was more decreased in ruptured urinary bladder cases {30.33 ± 1.33% (21 – 40%)} than in intact urinary bladder cases {32.80 ± 1.64% (19 - 40%)}. The overall mean ± SE lymphocyte percent of 31.44 ± 1.50% (19 - 40%) was also lower than the reported normal reference range for cattle. The peritoneal fluid neutrophil and lymphocyte percentages were lower than the blood ones. All the differences among the corresponding values of different groups were statistically ($p>0.05$) nonsignificant.

The overall mean ± SE monocyte/mesothelial cells/macrophage percent {27.4± 1.05% (19 - 40%)} was highly increased than the normal reference range (19.11%) reported for cattle. However, the monocyte/mesothelial cells/macrophage values were identical in both the groups of intact and ruptured urinary bladder cases, with the respective values of 27.66 ± 1.44 (19 – 39%) and 27.26 ± 0.71% (25 – 40%). A predominant decrease in eosinophil percentage, with more decrease in eosinophils in intact urinary bladder cases was observed during this study. The overall mean ± SE eosinophil percent was 7.34 ± 0.78% with 3 - 13% range. The mean ± SE eosinophil percent was 6.41 ± 0.76% (3 - 10%) and 8.09 ± 0.79% (3 - 13%) in intact and ruptured urinary bladder cases respectively. All the eosinophil values were lower than the normal values (47.50%) reported for cattle. But, the overall peritoneal fluid eosinophil percentage (7.34 ± 0.78%) was same as that of blood eosinophil percentage (7.4±0.50).

Polymorphonuclear - to - mononuclear cell percent ratio was same in whole group and intact urinary bladder cases i.e., 1:1.5, while it was lower (1:1.3) in ruptured urinary bladder cases. All the values were higher than the normal reference value (1:1).

Red blood corpuscles were totally absent in the peritoneal fluid samples obtained from intact urinary bladder cases except in one case where whole blood was obtained and discarded. However, in the peritoneal fluid samples obtained from ruptured urinary bladder cases, RBCs were found in abundance in 3 cases and very few in 2 cases (Fig.1).

The morphology and the presence of different cells in the peritoneal fluid samples varied according to the status of urinary bladder. In intact urinary bladder cases, neutrophils were mature and nondegenerate (Fig.2), while in ruptured urinary bladder cases degenerate and hypersegmented neutrophils (Fig.3) were more as compared to non-degenerate and band cells. The monocytes

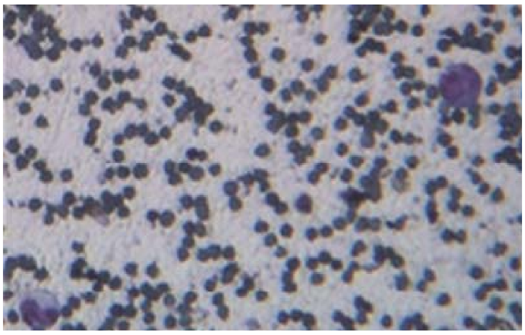


Fig.1 Slide made from the peritoneal fluid sample obtained from ruptured urinary bladder case showing abundant RBCs with few intact neutrophils and lymphocytes

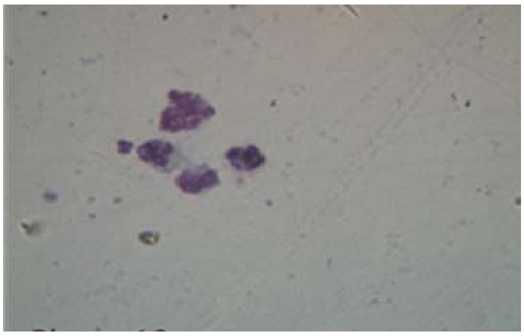


Fig.3 Slide made from the peritoneal fluid sample obtained from ruptured urinary bladder case showing degenerated and hypersegmented neutrophils.

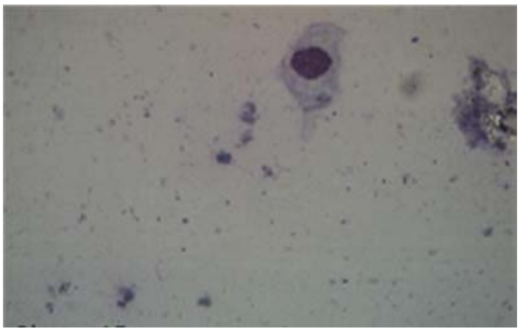


Fig.5 Slide made from the peritoneal fluid sample obtained from ruptured urinary bladder case showing mesothelial cell

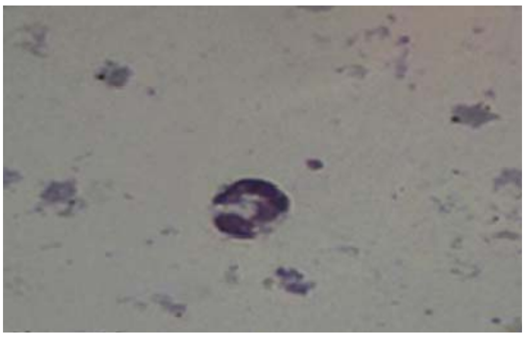


Fig.2 Slide made from the peritoneal fluid sample obtained from intact urinary bladder case showing intact neutrophils.

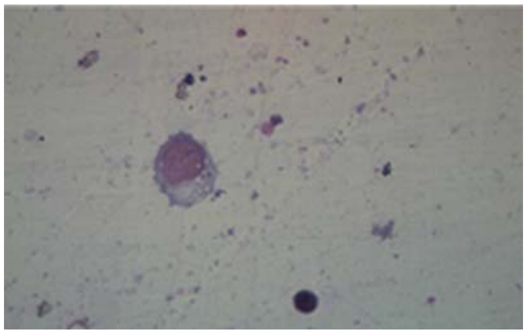


Fig.4 Slide made from the peritoneal fluid sample obtained from ruptured urinary bladder case showing activated monocyte with vacuolated cytoplasm

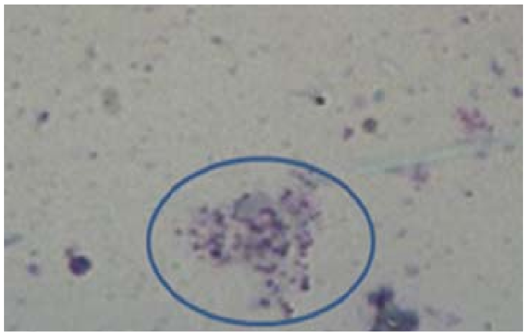


Fig.6 Slide made from the peritoneal fluid sample obtained from ruptured urinary bladder case showing bacteria extracellularly (within the circle)

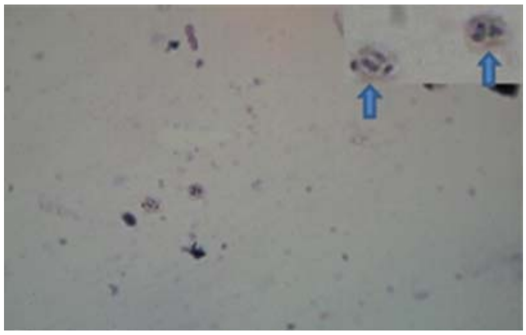


Fig.7 Slide made from the peritoneal fluid sample obtained from ruptured urinary bladder case showing bacteria intracellularly

were mildly activated as evidenced by foamy and vacuolated cytoplasm (Fig.4) in the peritoneal fluid samples of ruptured urinary bladder cases. Transitional epithelial cells were found only in the peritoneal fluid samples obtained from ruptured urinary bladder cases. Mesothelial cells were equally distributed in both the groups (Fig.5). Bacteria were found extracellularly (Fig.6) and intracelluarly in neutrophils in 4 cases with

ruptured urinary bladder and extracellularly in 2 cases with intact urinary bladder (Fig.7).

Discussion

Cytological analysis of the peritoneal fluid can help distinguish from fungal, bacterial, sterile, inflammatory or neoplastic causes of effusion, and often assists the clinician in initiating appropriate therapy while waiting for the results of culture, antimicrobial susceptibility testing and histological examination (Wilson *et al*., 1985; Roussel and Ward, 1985; Hirsch and Townsend, 1982; Oehme, 1969). When peritoneal fluid from clinically normal cattle is examined cytologically, mature nondegenerate neutrophils and macrophages predominate. Low numbers of small lymphocytes are expected (Al-Rukibat *et al*., 2006). Mesothelial cells commonly exfoliate into the cavity fluid of normal animals. Eosinophils are commonly seen in bovine peritoneal fluid (Wilson *et al*., 1985).

During this study increased percentage of neutrophils and decreased percentage of lymphocytes was recorded. Increased neutrophil percentage could be due to mild peritonitis in the clinical cases of obstructive urolithiasis induced by infiltration of peritoneal cavity with urine either because of rupture of urinary bladder or seepage of urine from highly distended intact urinary bladder, and due to reported cystocentesis performed in the field for diagnostic purposes. Decrease in lymphocyte percentage could be a compensatory phenomenon. Wilson *et al*., (1985) also recorded increased neutrophil percentage and compensatory decrease in lymphocyte percentage in the adult bovine peritonitis cases. There was no difference in neutrophil percentages between the cases with intact and ruptured urinary bladder. Additionally peritoneal fluid neutrophil and lymphocyte percentage was lower than that of blood. This indicates mild and localized nature of peritonitis in bovine. This could be attributed to the ability of this species to wall off areas of inflammation by fibrinous and fibrous adhesions (Jubb *et al.,* 1993). Secondly peritonitis in bovine is characterized by low cell counts as compared to highly nucleated cell counts of equine peritoneal fluid. This reflects the chronic course of peritonitis in cattle, but might also be the result of a less ability of this species to mobilize neutrophils from the bone marrow in response to inflammation (Radostits *et al*., 2000; Jain, 1986). The findings are also in consonance with those of Hirsch and Townsend, 1982, who reported only moderately elevated nucleated cell counts even in severe peritonitis in cattle.

The monocyte/mesothelial cell/macrophage percentage, though same in both the groups i.e., cases with intact urinary bladder and cases with ruptured urinary bladder, was highly increased than the normal reference range. This could be attributed to the fact that mesothelial cells commonly exfoliate into the cavity fluid of normal animals (Wilson *et al*., 1985), and during the obstructive urolithiasis, mesothelial cells and small lymphocytes may appear in large numbers.

A predominant decrease in eosinophil percentage was recorded in the clinical cases of obstructive urolithiasis in calves. This decrease was more profound in the cases with intact urinary bladder than that in the cases with ruptured urinary bladder. However the peritoneal fluid eosinophil percentage was same as that of blood. Eosinophils may be present in the peritoneal fluid of normal, non parasitized cattle; however increase in their number could be augmented by some pathological processes like nematodes of the genus Setaria in bovine abdomen (Wilson *et al*., 1985). With the increase in age, chances of infestation with different parasites also increase. The decreased eosinophil percentage of this study could thus be attributed to the younger age of the calves (median age 6.5 months). This could be substantiated by the findings of Anderson *et al*. (1995), who recorded significantly fewer eosinophils in the peritoneal fluid of calves than that in PF of cows. However, previous researchers have

Table: Mean ± SE and range of different cell count of peritoneal fluid.

Parameters		Whole group (N = 27)		Intact bladder (N = 12)		Ruptured bladder (N = 15)		Normal reference range
		Mean ± SE	Range	Mean ± SE	Range	Mean ± SE	Range	
Neutrophil (%)	Peritoneal fluid	30.48 ± 1.44	27 – 46	33.08 ± 1.39[a]	27 – 40	33.50± 1.48[a]	27 – 46	19.97[Ω]
	Blood	37.96 ± 0.86	30 – 46	38.67 ± 1.10[a]	31 – 46	37.27 ± 1.40[a]	30 – 46	¥28(15-45)
Lymphocyte (%)	Peritoneal fluid	31.44 ± 1.50	19 - 40	32.8 ± 1.64[a]	19 - 40	30.33 ± 1.33[a]	21 - 40	37.58[Ω]
	Blood	49.37 ± 0.92	39 – 60	49.87 ± 1.30[a]	43 – 59	49.26 ± 1.36[a]	39 - 60	¥58(45-75)
Monocyte Mesothelial cells/ Macrophages (%)	Peritoneal fluid	27.4 ± 1.05	19 – 40	27.66 ± 1.44[a]	19 – 39	27.26 ± 0.71[a]	25 –40	19.11[Ω]
	Blood (Monocytes only)	4.63 ± 0.30	1 – 7	4.13 ± 0.42[a]	1 – 6	5.13 ± 0.40[a]	3 – 7	¥4(2-7)
Eosinophils (%)	Peritoneal fluid	7.34 ± 0.78	3 – 13	6.41 ± 0.76[a]	3 – 10	8.09 ± 0.79[a]	3 –13	47.50[Ω]
	Blood	7.40 ± 0.50	2 – 11	7.00 ± 0.83[a]	2 – 11	7.80 ± 0.60[a]	3 – 11	¥9(2-20)
PMN:MN cells % ratio		1:1.5		1:1.5[a]		1:1.3[a]		1:1[¶]

[Ω] Wilson *et al.,* 1985 ¶ Radostits *et al.,* 2000 ¥ Sastry, 1983

Means in different groups bearing same superscript did not differ significantly at (P>0.05)

stated that eosinophils were uncommon in bovine peritoneal fluid (Oehme, 1969; Oehme and Noordsy, 1970; Hirsch and Townsend, 1982).

In earlier studies, two variables i.e. both eosinophils ≤ 10% and neutrophils ≥ 40% simultaneously were used for separation of cows with peritonitis and normal cows (Wilson *et al*., 1985). During this study eosinophil percentage ≤ 8% and neutrophil percentage ≥ 30% could be established as a reference range of peritonitis in calves. The lower percentage of eosinophils and neutrophils in calves could be established as criterion for diagnosis of peritonitis because of their lower percentage in peritoneal fluid than that in adult cattle (Anderson *et al*., 1995).

Polymorphonuclear-to-mononuclear cell percent ratio was same in whole group and intact urinary bladder cases i.e., 1:1.5, while it was lower (1:1.3) in ruptured urinary bladder cases. All the values were higher than the normal reference value (1:1). This could be attributed to the fact that the presence of urine within the peritoneal cavity incites a very mild inflammatory reaction, characterized by an increase in the proportion of polymorphonuclear cells (Wilson and Mac Williams, 1998).

Abundance of RBCs in the peritoneal fluid samples obtained from the ruptured urinary bladder cases could be due to bleeding from the rupture of cystic wall. The number of RBCs in the peritoneal fluid sample could vary according to the rent in the cystic wall. However, presence of whole blood in the peritoneal fluid sample from one case with intact urinary bladder could be due to the inadvertent rupture of the blood vessel. Peritoneal fluid from rest of the cases with intact urinary bladder was free from RBCs.

Status of urinary bladder profoundly affects the different cell type presence and their morphology in the peritoneal fluid samples. The presence of degenerate neutrophils and activated monocytes in the peritoneal fluid samples obtained from the ruptured urinary bladder cases could be because of the peritonitis induced by the presence of urine into the peritoneal cavity. Presence of mature and nondegenerate neutrophils in peritoneal fluid sample is a normal phenomenon (Wilson *et al*., 1985); however increase in their number indicates infection (Bohn and Callan, 2007). Transitional epithelial cells were found only in the peritoneal fluid samples obtained from ruptured urinary bladder cases, which could be possible only by the exposure of cystic epithelium to the peritoneal fluid following the rupture in cystic wall. Mesothelial cells were equally distributed in both the groups. Bacteria were found extracellularly as well as intracellularly in degenerate neutrophils in 4 cases with ruptured urinary bladder and only extracellularly in 2 cases with intact urinary bladder. Degenerate neutrophils and

intracellular and extracellular bacteria are indicative of a septic process, while as nondegenerate neutrophils and extracellular bacteria indicate contamination (Bohn and Callan, 2007). Sepsis could have been induced by reported diagnostic cystocentesis performed in the field and contamination of peritoneal fluid samples could be due to break in asepsis during abdominocentesis.

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Iranian Journal of Veterinary Science and Technology
Vol. 2, No. 1, 2010, 17-24

IJVST

سلول شناسی مایع صفاقی در موارد بالینی انسداد ناشی از سنگهای ادراری در گاو

جلال الدین پاراه، بشیر احمد مولوی، سجاد حسین، حکیم عطار، حمید الله ملک، مسعود سلیم میر، محمد مقبول درزی

دانشکده دامپزشکی و دامپروری، سرینگر، هند

دریافت مقاله: ۸۸/۹/۱۰ *پذیرش نهایی: ۸۹/۲/۱۵*

چکیده

۳۰ مورد بالینی از انسداد سیستم ادراری ناشی از تشکیل سنگ های ادراری، ۱۵ مورد بامثانه سالم و ۱۵ مورد با پارگی مثانه برای مطالعه تغییرات در سلول شناسی مایع صفاقی مورد استفاده قرار گرفتند. درصد نوتروفیل ها در مقایسه با مقدار مرجع گزارش شده برای گاو به شدت افزایش و درصد لنفوسیت کمی افزایش یافت. مقدار افزایش درصد نوتروفیل در هر دو گروه با مثانه سالم و مثانه پاره شده مشابه بود. درصد سلول های مونوسیت/مزوتلیال/ماکروفاژ نیز نسبت به مقدار مرجع به طور مشابه افزایش یافت. کاهش برجسته ای در درصد ائوزینوفیل همراه با کاهش بیشتر در گروه با مثانه سالم وجود داشت. نسبت سلول های پلی مرفونوکلئر به مونوسیت در هر دو گروه یکسان و بالاتر از مقدار مرجع طبیعی بود (۱:۱). تعداد ائوزینوفیل ها $\geq$ ۸ درصد و نوتروفیل ها $\leq$ ۳۰ درصد بود که می تواند به عنوان مقدار مرجع برای پریتونیت در گوساله ها قلمداد شود. گلبول های قرمز در نمونه های با مثانه سالم به جز در یک مورد وجود نداشت. با این وجود، در نمونه های مایع صفاقی به دست آمده از موارد با مثانه پاره شده گلبول های قرمز به مقدار فراوان در ۳ مورد و به تعداد اندک در دو مورد یافت شد. مرفولوژی و انواع مختلف سلولی موجود در نمونه های مایع صفاقی بر اساس وضعیت مثانه متفاوت بود. در موارد با مثانه سالم نوتروفیل ها بالغ و غیر دژنره بودند. در حالیکه در موارد با مثانه پاره شده نوتروفیل های دژنره با هسته های هیپرپیگمانته بیشتر بودند. انتشار سلول های مزوتلیال در هر دو گروه مشابه بود. باکتری ها به صورت خارج سلولی و نیز داخل سلولی در نوتروفیل های دژنره در ۴ مورد از موارد با مثانه پاره شده و تنها به صورت خارج سلولی در ۲ مورد ازمثانه های سالم یافت شد. سلول شناسی مایع صفاقی می تواند برای تمایز پریتونیت از موارد سالم و یا پریتونیت غیر سپتیک از پریتونیت سپتیک علاوه بر تشخیص یورو پریتونئوم در گوساله ها به کار رود.

واژگان کلیدی: گوساله، سیتولوژی، آزمایش مایع صفاقی، سنگ ادراری